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(54) RECOMBINANT ANTIBODIES SPECIFIC FOR TNF alpha

REKOMBINANTE ANTIKÖRPER GEGEN TNF-ALPHA

ANTICORPS DE RECOMBINAISON SPECIFIQUE AU FNT alpha

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(56) References cited:  
EP-A- 0 355 067 EP-A- 0 403 156  
WO-A-91/09967

- Journal of Infectious Diseases, vol. 161, no. 6, June 1990, (Chicago, US), S.OPAL et al.: "Efficacy of a monoclonal antibody directed against tumor necrosis factor in protecting neutropenic rats from lethal infection with Pseudomonas aeruginosa", pages 1148-1152, see the whole article
- Proceedings of the National Academy of Sciences of USA, vol. 86, no. 24, December 1989, (Washington, US), C. QUEEN et al.: "A humanized antibody that binds to the interleukin 2 receptor", pages 10029-10033, see page 10033, left-hand column, lines 10-45 (cited in the application)

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**D scription****Fi ld of the Invention**

5 This invention relates to recombinant, in particular humanised, antibody molecules having specificity for antigenic determinants of tumour necrosis factor alpha (TNF- $\alpha$ ), to processes for their production using recombinant DNA technology, and to their therapeutic uses.

For the purposes of the present description the term "recombinant antibody molecule" is used to describe an antibody molecule produced by any process involving the use of recombinant DNA technology, including any analogues of natural immunoglobulins or their fragments.

Also for the purposes of the present description the term "humanised antibody molecule" is used to describe a molecule having an antigen binding site derived from an immunoglobulin from a non-human species, and remaining immunoglobulin derived parts of the molecule being derived from a human immunoglobulin. Thus humanised antibody molecules include humanised chimeric antibody molecules comprising complete non-human heavy and/or light chain variable region domains linked to human constant region domains. Humanised antibody molecules also comprise CDR-grafted humanised antibody molecules comprising one or more CDRs from a non-human antibody grafted into a heavy and/or light chain human variable region framework.

The antigen binding specificity of antibodies is determined by their complementarily determining regions (CDRs) which are relatively short peptide sequences carried on the framework regions of the variable domains. There are 3CDRs, (CDR1, CDR2 and CDR3) in each of the heavy and light chain variable domains.

The abbreviation "MAb" is used to indicate a monoclonal antibody. In the present description reference is made to a number of publications by number, and these publications are listed in numerical order at the end of the description.

**Background of the Invention**

25 Natural immunoglobulins have been known for many years, as have the various fragments thereof, such as the Fab, Fv, (Fab')<sub>2</sub> and Fc fragments, which can be derived by enzymatic cleavage. Natural immunoglobulins comprise a generally Y-shaped molecule having an antigen-binding site towards the end of each upper arm. The remainder of the structure, and particularly the stem of the Y, mediates the effector functions associated with immunoglobulins.

30 Natural immunoglobulins have been used in assay, diagnosis and, to a more limited extent, therapy. However, such uses, especially in therapy, were hindered until recently by the polyclonal nature of natural immunoglobulins. A significant step towards the realisation of the potential of immunoglobulins as therapeutic agents was the discovery of procedures for the reproducible production of monoclonal antibodies (MAbs) of defined specificity (1).

However, most MAbs are produced by hybridomas which are fusions of rodent spleen cells with rodent myeloma cells. They are therefore essentially rodent proteins. There are very few reports of the production of human MAbs.

35 Since most available MAbs are of rodent origin, they are naturally antigenic in humans and thus can give rise to an undesirable immune response termed the HAMA (Human Anti-Mouse Antibody) response if the MAb is administered to a human. Therefore, the use of rodent MAbs as therapeutic agents in humans is inherently limited by the fact that the human subject will mount an immunological response to the MAb and will either remove it entirely or at least reduce its effectiveness. In practice, MAbs of rodent origin may not be used in patients for more than one or a few treatments as a HAMA response soon develops rendering the MAb ineffective as well as giving rise to undesirable reactions. For instance, OKT3 a mouse IgG2a/k MAb which recognises an antigen in the T-cell receptor-CD3 complex has been approved for use in many countries throughout the world as an immunosuppressant in the treatment of acute allograft rejection [Chatenoud *et al* (2) and Jeffers *et al* (3)]. However, in view of the rodent nature of this and other such MAbs, a significant HAMA response which may include a major anti-idiotypic component, may build up on use. Clearly, it would be highly desirable to diminish or abolish this undesirable HAMA response and thus enlarge the areas of use of such antibodies.

Proposals have therefore been made to render non-human MAbs less antigenic in humans. Such techniques can be generically termed "humanisation" techniques. These techniques typically involve the use of recombinant DNA technology to manipulate DNA sequences encoding the polypeptide chains of the antibody molecule.

40 Early methods for humanising MAbs involved production of chimeric antibodies in which an antigen binding site comprising the complete variable domains of one antibody is linked to constant domains derived from another antibody. Methods for carrying out such chimerisation procedures are described in EP0120694 (Celltech Limited), EP0125023 (Genentech Inc. and City of Hope), EP-A-0 171496 (Res. Dev. Corp. Japan), EP-A-0 173 494 (Stanford University), and WO 86/01533 (Celltech Limited). These prior patent applications generally disclose processes for preparing antibody molecules having the variable domains from a mouse MAb and the constant domains from a human immunoglobulin. Such humanised chimeric antibodies, however, still contain a significant proportion of non-human amino acid sequence, i.e. the complete non-human variable domains, and thus may still elicit some HAMA response, particularly if administered

over a prolonged period [Begent *et al* (ref. 4)].

In an alternative approach, described in EP-A-0239400 (Winter), the complementarity determining regions (CDRs) of a mouse MAb have been grafted onto the framework regions of the variable domains of a human immunoglobulin by site directed mutagenesis using long oligonucleotides. Such CDR-grafted humanised antibodies are much less likely to give rise to a HAMA response than humanised chimeric antibodies in view of the much lower proportion of non-human amino acid sequence which they contain.

The earliest work on humanising MAbs by CDR-grafting was carried out on MAbs recognising synthetic antigens, such as the NP or NIP antigens. However, examples in which a mouse MAb recognising lysozyme and a rat MAb recognising an antigen on human T-cells were humanised by CDR-grafting have been described by Verhoeyen *et al* (5) and Riechmann *et al* (6) respectively. The preparation of CDR-grafted antibody to the antigen on human T cells is also described in WO 89/07452 (Medical Research Council).

In Riechmann *et al*/Medical Research Council it was found that transfer of the CDR regions alone [as defined by Kabat refs. (7) and (8)] was not sufficient to provide satisfactory antigen binding activity in the CDR-grafted product. Riechmann *et al* found that it was necessary to convert a serine residue at position 27 of the human heavy chain sequence to the corresponding rat phenylalanine residue to obtain a CDR-grafted product having improved antigen binding activity. This residue at position 27 of the heavy chain is within the structural loop adjacent to CDR1. A further construct which additionally contained a human serine to rat tyrosine change at position 30 of the heavy chain did not have a significantly altered binding activity over the humanised antibody with the serine to phenylalanine change at position 27 alone. These results indicated that changes to residues of the human sequence outside the CDR regions, in particular in the structural loop adjacent to CDR1 of the heavy chain, may be necessary to obtain effective antigen binding activity for CDR-grafted antibodies which recognise more complex antigens. Even so the binding affinity of the best CDR-grafted antibodies obtained was still significantly less than the original MAb.

Recently Queen *et al* (9) have described the preparation of a humanised antibody that binds to an interleukin 2 receptor, by combining the CDRs of a murine MAb (anti-Tac) with human immunoglobulin framework and constant regions. The human framework regions were chosen to maximise homology with the anti-Tac MAb sequence. In addition computer modelling was used to identify framework amino acid residues which were likely to interact with the CDRs or antigen, and mouse amino acids were used at these positions in the humanised antibody.

In WO90/07861 Queen *et al* propose four criteria for designing humanised immunoglobulins. The first criterion is to use as the human acceptor the framework from a particular human immunoglobulin that is unusually homologous to the non-human donor immunoglobulin to be humanised, or to use a consensus framework from many human antibodies. The second criterion is to use the donor amino acid rather than the acceptor if the human acceptor residue is unusual and the donor residue is typical for human sequences at a specific residue of the framework. The third criterion is to use the donor framework amino acid residue rather than the acceptor at positions immediately adjacent to the CDRs. The fourth criterion is to use the donor amino acid residue at framework positions at which the amino acid is predicted to have a side chain atom within about 3 Å of the CDRs in a three-dimensional immunoglobulin model and to be capable of interacting with the antigen or with the CDRs of the humanised immunoglobulin. It is proposed that the second, third or fourth criteria may be applied in addition or alternatively to the first criterion, and may be applied singly or in any combination.

WO90/07861 describes in detail the preparation of a single CDR-grafted humanised antibody, a humanised antibody having specificity for the p55 Tac protein of the IL-2 receptor. The combination of all four criteria, as above, were employed in designing this humanised antibody, the variable region frameworks of the human antibody EU (7) being used as acceptor. In the resultant humanised antibody the donor CDRs were as defined by Kabat *et al* (7 and 8) and in addition the mouse donor residues were used in place of the human acceptor residues, at positions 27, 30, 48, 66, 67, 89, 91, 94, 103, 104, 105 and 107 in the heavy chain and at positions 48, 60 and 63 in the light chain, of the variable region frameworks. The humanised anti-Tac antibody obtained is reported to have an affinity for p55 of  $3 \times 10^9 \text{ M}^{-1}$ , about one-third of that of the murine MAb.

We have further investigated the preparation of CDR-grafted humanised antibody molecules and have identified a hierarchy of positions within the framework of the variable regions (i.e. outside both the Kabat CDRs and structural loops of the variable regions) at which the amino acid identities of the residues are important for obtaining CDR-grafted products with satisfactory binding affinity. This has enabled us to establish a protocol for obtaining satisfactory CDR-grafted products which may be applied very widely irrespective of the level of homology between the donor immunoglobulin and acceptor framework. The set of residues which we have identified as being of critical importance overlaps but does not coincide with the residues identified by Queen *et al* (9). Our copending International patent application WO91/09967 describes this protocol for the preparation of CDR-grafted, in particular humanised, antibody heavy and light chains and complete molecules of any desired specificity. The full disclosure of International patent application WO91/09967 is incorporated in the present description by reference.

Tempest *et al* (10) have very recently described the preparation of a reshaped human monoclonal antibody for use in inhibiting human respiratory syncytial virus (RSV) infection *in vivo*. This reshaped antibody was prepared by grafting

synthetic oligo nucleotides coding for the CDRs of a murine MAb, which neutralises RSV infection, by site - directed mutagenesis into DNA coding for the frameworks of a human IgG1, monoclonal antibody. However the simple reshaped antibody in which the CDRs alone had been transferred between mouse and human antibodies had only very poor binding for RSV which was not significantly above background. In order to partially restore binding ability it proved  
 5 necessary to additionally convert human residues to mouse residues in a framework region adjacent to CDR3 of the heavy chain. Tempest *et al* did not convert human residues to mouse residues at important positions identified in the protocol of WO91/09967.

TNF $\alpha$  is a cytokine which is released by and interacts with cells of the immune system. Thus TNF $\alpha$  is released by macrophages which have been activated by lipopolysaccharide (LPS) of gram negative bacteria. As such TNF $\alpha$  appears  
 10 to be an endogenous mediator of central importance involved in the development and pathogenesis of endotoxic shock associated with bacterial sepsis. Antibodies to TNF $\alpha$  has been proposed for the prophylaxis and treatment of endotoxic shock (Beutler *et al* (11)). However the antibodies to TNF $\alpha$  currently available for use in such treatment are typically murine MABs. As such these murine MABs are of only limited use for treatment of humans in view of the undesirable HAMA (Human Anti-Mouse Antibody) response which they can elicit if used for more than one or a few treatments. It is  
 15 thus a highly desirable objective to prepare humanised anti-TNF $\alpha$  products for use in human therapy.

Our co-pending International patent application WO91/09967 describes, among other things, the preparation of humanised CDR-grafted antibody products which have specificity for TNF $\alpha$ . In particular WO91/09967 describes, in Example 5, preparation of specific humanised CDR grafted antibodies to human TNF $\alpha$  derived from the murine anti-human TNF $\alpha$  MABs identified as 61E71 (alternatively known as CB0006), hTNF1 (alternatively known as CB0010), hTNF3  
 20 and 101.4. The present application relates specifically to recombinant, in particular humanised antibodies to human TNF $\alpha$ , including those described in WO91/09967 and further improved humanised CDR-grafted antibodies to human TNF $\alpha$  based upon the hTNF1 (CB0010) and 101.4 murine MABs. Further studies of various anti-human TNF $\alpha$  murine MABs have revealed that hTNF1 and 101.4 have particularly desirable properties for use in anti-TNF therapy.

The present invention provides an antibody molecule which has specificity for human TNF $\alpha$  comprising a composite  
 25 heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising predominantly human acceptor antibody heavy chain framework residues and donor antibody heavy chain antigen-binding residues, said donor antibody having specificity for said human TNF $\alpha$ , wherein, according to the Kabat numbering system, in said composite heavy chain, amino acid residues 23, 24, 31 to 35, 49 to 65 and 95 to 100 at least are donor residues.

30 Preferably, in the antibody molecule, amino acid residues 71, 73 and 78 in said composite heavy chain are additionally donor residues.

If desired, in the antibody molecule, amino acid residues 6, 37, 48 and 94 are additionally donor residues.

If further desired, in the antibody molecule, amino acid residues 2, 4, 25, 36, 39, 47, 93, 103, 104, 106 and 107 are additionally donor residues.

35 Optionally, in the antibody molecule, amino acid residues 26 to 30 are additionally donor residues.

The present invention also provides an antibody molecule which has specificity for human TNF $\alpha$  comprising a composite light chain and a complementary heavy chain, said composite light chain having a variable domain comprising predominantly human acceptor antibody light chain framework residues and donor antibody light chain antigen-binding  
 40 residues, said donor antibody having specificity for said human TNF $\alpha$ , wherein, according to the Kabat numbering system, in said composite light chain, amino acid residues 24 to 34, 46, 47, 50 to 56 and 89 to 97 at least are donor residues.

The present invention further provides an antibody molecule which has specificity for human TNF $\alpha$  comprising a composite light chain and a complementary heavy chain, said composite light chain having a variable domain comprising predominantly human acceptor antibody light chain framework residues and donor antibody light chain antigen-binding  
 45 residues, said donor antibody having specificity for said human TNF $\alpha$ , wherein, according to the Kabat numbering system, in said composite light chain, amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97 at least are donor residues.

In the antibody molecule of the first aspect of the invention preferably the complementary light chain is a composite light chain, said composite light chain having a variable domain comprising predominantly human-acceptor antibody light chain framework residues and donor antibody light chain antigen-binding residues, wherein, according to the Kabat  
 50 numbering system, in said composite light chain, amino acid residues 24 to 34, 46, 47, 50 to 56 and 89 to 97 at least are donor residues.

In the antibody molecule of the first aspect of the invention, alternatively, the complementary light chain is a composite light chain, said composite light chain having a variable domain comprising predominantly human acceptor antibody light chain framework residues and donor antibody light chain antigen-binding residues wherein, according to the  
 55 Kabat numbering system, in said composite light chain, amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97 at least are donor residues.

In the antibody molecule as defined above, preferably amino acid residues 2, 4, 6, 35, 36, 38, 44, 47, 49, 62, 64 to 69, 85, 87, 98, 99, 101 and 102 in said light chain are additionally donor residues.

In the antibody molecule as defined above, optionally amino acid residues 1, 3, 10 to 12, 21, 37, 40, 45, 60, 63, 70, 73, 83, 103 and 105 in said light chain are additionally donor residues.

In the antibody molecule as defined above, the donor antibody is preferably a murine monoclonal antibody selected from the group consisting of CB006 (alternatively known as 61E71); CB0010 (alternatively known as hTNF1); hTNF3; and 101.4.

The present invention also provides a therapeutic composition comprising the antibody molecule as defined above in combination with a pharmaceutically acceptable carrier.

The present invention also provides the antibody molecule or the therapeutic composition as defined above for use in a therapeutic method comprising administering the antibody to a human subject in an amount effective to neutralize TNF $\alpha$ .

The present invention also provides the antibody molecule or the therapeutic composition as defined above for use in treating rheumatoid arthritis.

The recombinant antibody molecules of the invention are preferably TNF $\alpha$  neutralising, i.e. are capable of reducing or inhibiting a biological activity of human TNF $\alpha$  as measured by an *in vitro* or *in vivo* test.

Preferably, the antigen binding regions of the heavy chain variable domain comprise CDRs corresponding to the Kabat CDRs at CDR2 (residues 50-65) and CDR3 (residues 95-102) and a composite of the Kabat and structural loop CDRs at CDR1 (residues 26-35), i.e. residues 26-30 are included within CDR1.

The residue designations given above and elsewhere in the present application are numbered according to the Kabat numbering [refs. (7) and (8)]. Thus the residue designations do not always correspond directly with the linear numbering of the amino acid residues. The actual linear amino acid sequence may contain fewer or additional amino acids than in the strict Kabat numbering corresponding to a shortening of, or insertion into, a structural component, whether framework or CDR, of the basic variable domain structure. For example, the heavy chain variable region of the anti-Tac antibody described by Queen *et al* (9) contains a single amino acid insert (residue 52a) after residue 52 of CDR2 and a three amino acid insert (residues 82a, 82b and 82c) after framework residue 82, in the Kabat numbering. The correct Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

It will be appreciated that when the antibody molecules of the invention, as described above and elsewhere in the present description, are applied to a particular donor/acceptor antibody pair, in some cases the donor and acceptor amino acid residues may be identical at a particular position identified for change to the donor residue, and thus no change of acceptor framework residue is required.

The antigen binding regions of the composite light chain variable domain comprise CDRs corresponding to the Kabat CDRs at CDR1 (residue 24-34), CDR2 (residues 50 to 56) and CDR3 (residues 89-97).

Preferably, the heavy chain variable domain comprises human acceptor framework (especially EU human acceptor framework) and hTNF1 donor antigen binding regions wherein the framework comprises hTNF1 donor residues at positions 12, 27, 30, 38, 46, 48, 66, 67, 69, 71, 73, 76, 83, 89, 91 and 94.

The EU heavy framework has residues in framework 4 (FR4) of the heavy chain which are anomalous for human heavy chain frameworks. Thus preferably human consensus residues are used in place of EU residues in FR4 of the heavy chain. In particular, the human consensus residue threonine (T) may be used at position 108. Fortuitously the murine hTNF1 residue at position 108 is also threonine.

Preferably, the light chain variable domain comprises human acceptor framework (especially EU human acceptor framework) and hTNF1 donor antigen binding regions wherein the framework comprises hTNF1 donor residues at positions 3, 42 and 49.

When the EU human framework is used for the light chain it is also desirable to change residues from EU residues at positions 48, 83, 106 and 108, as the EU residues at these positions are anomalous for human antibodies. Thus the human consensus residues may be used at some or preferably all of these residues, i.e. isoleucine (I) at position 48, valine (V) at position 83, isoleucine (I) at position 106 and arginine (R) at position 108. Fortuitously the murine hTNF1 residues are the same as the human consensus residues at positions 48 (I), 106 (I) and 108 (R). However, the human consensus residue valine (V) at position 83 differs from both the EU residue (F) and the hTNF1 residue (L) at this position.

In an alternative preferred embodiment, the heavy chain domain comprises human acceptor framework (especially KOL human acceptor framework) and 101.4 donor antigen binding regions wherein the framework comprises 101.4 donor residues at positions 4, 11, 23, 24, 28, 73, 77, 78, 79, 91, 93 and 94.

The KOL residue proline (P) at position 108 of the heavy chain is anomalous for human antibodies. Thus preferably the human consensus residue leucine (L) is at this position if KOL is used as the human acceptor framework. Fortuitously the murine 101.4 antibody has the human consensus residue (L) at this position.

Preferably, in this alternative aspect, the light chain variable domain comprises human acceptor framework (especially REI human acceptor framework) and 101.4 donor residues at positions 1, 3, 4 and 73.

The REI light chain human framework has residues which are anomalous for human antibodies at positions 39 (threonine, T), 104 (leucine, L) 105 (glutamine, Q), and 107 (threonine, T). Thus when REI is used as the light chain

framework, human consensus residues are used at positions 39 (lysine, K), 104 (valine, V), 105 (glutamic acid, E) and 107 (lysine, K). Fortuitously the murine 101.4 residues are the same as the human consensus residues at positions 39 (K), 105 (E) and 107 (K). However, the human consensus residue at position 104 (V) differs from the leucine (L) REI and murine 101.4 residues at this position.

The present invention also includes antibody fragments, such as a Fab, Fab', F(ab')<sub>2</sub> or Fv fragment.

Also the antibody molecules of the present invention may have attached to them an effector or reporter molecule. For instance, it may have a macrocycle, for chelating a heavy metal atom, or a toxin, such as ricin, attached to it by a covalent bridging structure. Alternatively, the procedures of recombinant DNA technology may be used to produce an immunoglobulin molecule in which the Fc fragment or CH3 domain of a complete immunoglobulin molecule has been replaced by, or has attached thereto by peptide linkage, a functional non-immunoglobulin protein, such as an enzyme or toxin molecule.

The amino acid sequences of the heavy and light chain variable domains of the CB0010, 101.4, CB0006 and hTNF3 murine MABs, CDR-grafted variants thereof and human acceptor antibodies are given in the accompanying diagrams Figures 1, 2, 3 and 4 respectively. The recombinant and humanised antibody products of the invention may be prepared using recombinant DNA techniques, for instance substantially as described in WO91/09967.

Any appropriate human acceptor variable region framework sequences may be used having regard to class/type of the donor antibody from which the antigen binding regions are derived. Preferably, the type of human acceptor framework used is of the same/similar class/type as the donor antibody. Conveniently, the framework may be chosen to maximise/optimize homology with the donor antibody sequence particularly at positions close or adjacent to the CDRs. However, a high level of homology between donor and acceptor sequences is not critical for application of the present invention. The present invention identifies a hierarchy of framework residue positions at which donor residues may be important or desirable for obtaining a CDR-grafted antibody product having satisfactory binding properties. The CDR-grafted products usually have binding affinities of at least 10<sup>5</sup> M<sup>-1</sup>, preferably at least about 10<sup>8</sup> M<sup>-1</sup>, or especially in the range 10<sup>8</sup>-10<sup>12</sup> M<sup>-1</sup>. In principle, the present invention is applicable to any combination of anti-hTNF $\alpha$  donor and human acceptor antibodies irrespective of the level of homology between their sequences. Examples of human frameworks which may be used are KOL, NEWM, REI, EU, TUR, TEI, LAY and POM (refs. 7 and 8) and the like; for instance KOL and NEWM for the heavy chain and REI for the light chain and EU, LAY and POM for both the heavy chain and the light chain.

Also the constant region domains of the products of the invention may be selected having regard to the proposed function of the antibody in particular the effector functions which may be required. For example, the constant region domains may be human IgA, IgE, IgG or IgM domains. In particular, IgG human constant region domains may be used, especially of the IgG1 and IgG3 isotypes, when the humanised antibody molecule is intended for therapeutic uses, and antibody effector functions are required. Alternatively, IgG2 and IgG4 isotypes may be used when the humanised antibody molecule is intended for therapeutic purposes and antibody effector functions are not required, e.g. for simple blocking of TNF activity.

However, the remainder of the antibody molecules need not comprise only protein sequences from immunoglobulins. For instance, a gene may be constructed in which a DNA sequence encoding part of a human immunoglobulin chain is fused to a DNA sequence encoding the amino acid sequence of a functional polypeptide such as an effector or reporter molecule.

The general methods by which the vectors may be constructed, transfection methods and culture methods are well known per se. Such methods are shown, for instance, in references 12 and 13.

The DNA sequences which encode the anti-hTNF $\alpha$  antibody molecule amino acid sequences may be obtained by methods well known in the art. For example the anti-TNF coding sequences may be obtained by genomic cloning, or cDNA cloning from suitable anti-hTNF $\alpha$  producing hybridoma cell lines. Positive clones may be screened using appropriate probes for the heavy and light chain genes in question. Also PCR cloning may be used. DNA sequence coding for part or all of the antibody heavy and light chains may be synthesised as desired from the determined DNA sequence or on the basis of the corresponding amino acid sequence.

DNA coding for acceptor, e.g. human acceptor, sequences may be obtained in any appropriate way. For example DNA sequences coding for preferred human acceptor frameworks such as KOL, REI, EU and NEWM, are widely available to workers in the art, or may be readily synthesised on the basis of their known amino acid sequences (see refs. 7 & 8).

The standard techniques of molecular biology may be used to prepare DNA sequences coding for the chimeric and CDR-grafted humanised antibody products. Desired DNA sequences may be synthesised completely or in part using oligonucleotide synthesis techniques. Site-directed mutagenesis and polymerase chain reaction (PCR) techniques may be used as appropriate. For example oligonucleotide directed synthesis as described by Jones et al (ref. 14) may be used. Also oligonucleotide directed mutagenesis of a pre-existing variable region as, for example, described by Verhoeyen et al (ref. 5) or Riechmann et al (ref. 6) may be used. Also enzymatic filling in of gapped oligonucleotides using T<sub>4</sub> DNA polymerase as, for example, described by Queen et al (ref. 9) may be used.

Any suitable host cell/vector system may be used for expression of the DNA sequences coding for the recombinant,

chimeric and CDR-grafted humanised antibody heavy and light chains. Bacterial e.g. *E. coli*, and other microbial systems may be used, in particular for expression of antibody fragments such as Fab and F(ab')<sub>2</sub> fragments, and especially Fv fragments and single chain antibody fragments e.g. single chain Fvs. Eucaryotic e.g. mammalian host cell expression systems may be used for production of larger CDR-grafted antibody products, including complete antibody molecules, and/or if glycosylated products are required. Suitable mammalian host cells include CHO cells and myeloma or hybridoma cell lines.

The invention also includes therapeutic and diagnostic compositions comprising the recombinant and humanised antibody products of the invention and the uses of these products and the compositions in therapy and diagnosis.

Thus in a further aspect the invention provides a therapeutic or diagnostic composition comprising a recombinant or humanised antibody according to the invention in combination with a pharmaceutically acceptable excipient, diluent or carrier.

The recombinant or humanised antibody may be the sole active ingredient in the therapeutic or diagnostic composition or may be accompanied by one or more other active ingredients including other antibody ingredients, e.g. anti-T cell, anti-IFN $\gamma$ , or anti-LPS antibodies, or non-antibody ingredients such as xanthines. The therapeutic and diagnostic compositions may be in unit dosage form, in which case each unit dose comprises an effective amount of the recombinant or humanised antibody of the invention.

The antibodies and compositions may be utilised in any therapy where it is desired to reduce the level of TNF present in the human or animal body. The TNF may be in circulation in the body or present in an undesirably high level localised at a particular site in the body.

For example, elevated levels of TNF are implicated in immunoregulatory and inflammatory disorders and in septic, or endotoxic, and cardiovascular shock. The antibody or composition may be utilised in therapy of conditions which include sepsis, septic or endotoxic shock, cachexia, adult respiratory distress syndrome, AIDS, allergies, psoriasis, T.B., inflammatory bone disorders, blood coagulation disorders, burns, rejection episodes following organ or tissue transplant and autoimmune disease e.g. organ specific disease such as thyroiditis or non-specific organ diseases such as rheumatoid and osteo-arthritis.

Additionally, the antibody or composition may be used to ameliorate side effects associated with TNF generation during neoplastic therapy and also to eliminate or ameliorate shock related symptoms associated with the treatment or prevention of graft rejection by use of an antilymphocyte antibody, or may be used for treating multi-organ failure (MOF).

The recombinant and humanised antibodies and compositions of the invention are preferably for treatment of sepsis or septic/endotoxic shock.

The antibodies and compositions may be for administration in any appropriate form and amount according to the therapy in which they are employed. This may be for prophylactic use, for example where circumstances are such that an elevation in the level of TNF might be expected or alternatively, they may be for use in reducing the level of TNF after it has reached an undesirably high level or as the level is rising.

The therapeutic or diagnostic composition may take any suitable form for administration, and, preferably is in a form suitable for parenteral administration e.g. by injection or infusion, for example by bolus injection or continuous infusion. Where the product is for injection or infusion, it may take the form of a suspension, solution or emulsion in an oily or aqueous vehicle and it may contain formulatory agents such as suspending, preservative, stabilising and/or dispersing agents.

Alternatively, the antibody or composition may be in dry form, for reconstitution before use with an appropriate sterile liquid.

If the antibody or composition is suitable for oral administration, e.g. in the case of antibody fragments, the formulation may contain, in addition to the active ingredient, additives such as: starch - e.g. potato, maize or wheat starch or cellulose - or starch derivatives such as microcrystalline cellulose; silica; various sugars such as lactose; magnesium carbonate and/or calcium phosphate. It is desirable that, if the formulation is for oral administration it will be well tolerated by the patient's digestive system. To this end, it may be desirable to include in the formulation mucus formers and resins. It may also be desirable to improve tolerance by formulating the antibody or compositions in a capsule which is insoluble in the gastric juices. It may also be preferable to include the antibody or composition in a controlled release formulation.

The antibody molecules and fragments of the present invention will be of use in a method of treatment of a human or animal subject suffering from or at risk of a disorder associated with an undesirably high level of TNF, the method comprising administering to the subject an effective amount of the antibody or composition of the invention. In particular, the human or animal subject may be suffering from, or at risk from, sepsis, or septic or endotoxic shock.

The dose at which the antibody is administered depends on the nature of the condition to be treated, the degree to which the TNF to be neutralised is, or is expected to be, raised above a desirable level, and on whether the antibody is being used prophylactically or to treat an existing condition. The dose will also be selected according to the age and conditions of the patient.

Thus, for example, where the product is for treatment or prophylaxis of septic shock suitable doses of antibody to TNF lie in the range 0.001-30mg/kg/day, preferably 0.01-10mg/kg/day and particularly preferably 0.1-2mg/kg/day.

The antibody products may be used in diagnosis e.g. in in vivo diagnosis and imaging of disease states involving elevated TNF levels.

The invention is further described by way of illustration only in the following Examples which refers to the accompanying diagrams, Figures 1 - 6.

### **Brief Description of the Figures**

Figure 1 shows amino acid sequences for the variable domains of the heavy and light chains for the human acceptor antibody EU (1EU), the murine MAb CB0010 (*h t n f 1*) and humanised CDR grafted light (gEU) and heavy (2hEUg) chains;

Figure 2 shows amino acid sequences for the variable region domains of the human acceptor antibodies RE1 (*1 r e 1*) for the light chain and KOL (KOL) for the heavy chain, of the heavy and light chains of the murine MAb 101.4 (101/4) and humanised grafted light and heavy chains (both designated g1014);

Figure 3 shows amino acid sequences for the variable region domains of the human acceptor antibodies RE1 (RE1) for the light chain and KOL (KOL) for the heavy chain, of the heavy and light chains of the murine MAb CB0006 (CB6) and humanised grafted light and heavy chains (both designated gCB6);

Figure 4 shows amino acid sequences for the variable region domains of the human acceptor antibodies RE1 (RE1) for the light chain and KOL (KOL) for the heavy chain, of the heavy (HTNF3) and light (hTNF3) chains of the murine MAb HTNF3 and humanised grafted light (gHTNF3) and heavy (ghTNF3) chains;

Figure 5 shows a graph comparing the ability of murine CB0010 (hTNF1) and CDR-grafted CB0010 (GrhTNF1; CDP571) to compete with HRP-conjugated murine HTNF1 for binding to recombinant human TNF $\alpha$ , and

Figure 6 shows a graph comparing the ability of murine HTNF1 (CB0010) and CDR-grafted HTNF1 (CP571) to neutralise recombinant TNF $\alpha$  in the L929 bioassay.

### **Detailed Description of Embodiments of the Invention**

#### **Example 1**

#### **CDR-Grafting of murine anti-TNF $\alpha$ antibodies**

A number of murine anti-human TNF $\alpha$  MAbs were CDR-grafted substantially as described in detail in WO91/09967 for the CDR-grafting of the murine anti-CD3 antibody OKT3. In this and subsequent Examples, the chimeric and CDR-grafted humanised antibodies were prepared using human IgG4 constant region domains, substantially as described for preparation of  $\gamma$ 4 chimeric and CDR-grafted OKT3 antibodies in WO91/09967. It will be appreciated, however, that human constant region domains of other types and isotypes, e.g. IgG1, IgG2 and IgG3, could also have been used without significantly altering the procedures described.

These anti-hTNF $\alpha$  antibodies included the murine MAbs designated CB0006 (also known as 61E71), CB0010 (also known as hTNF1), hTNF3 and 101.4 A brief summary of the CDR-grafting of each of these antibodies is given below.

#### **CB0006**

A similar analysis as described in Example 1, Section 12.1. of WO91/09967 was carried out for CB0006 and for the heavy chain 10 framework residues were identified at positions 23, 24, 48, 49, 68, 69, 71, 73, 75 and 88 as residues to potentially retain as murine. The human frameworks chosen for CDR-grafting of this antibody, and the hTNF3 and 101.4 antibodies were RE1 for the light chain and KOL for the heavy chain. The amino acid sequences of the murine CB0006 (CB6) (heavy and Light) RE1 (RE1) light and KOL (KOL) heavy chain variable domains are given in Figure 3.

Three genes were built, the first of which coded for amino acid residues 23, 24, 48, 49, 71 and 73 [gH341(6)] as murine residues. The amino acid sequence of the variable domain coded by this first gene is shown as gCB6 in the heavy chain summary in Figure 3. The second gene also had amino acid residues 75 and 88 as murine residues [gH341(8)] while the third gene additionally had amino acid residues 68, 69, 75 and 88 as murine residues [gH341(10)]. Each was co-expressed with gL221, the minimum grafted light chain (CDRs only) shown as gCB6 in the heavy chain summary in Figure 3. The gL221/gH341(6) and gL221/gH341(8) antibodies both bound as well to TNF as murine 61E71. The gL221/gH341(10) antibody did not express and this combination was not taken further.



Subsequently the gL221/gH341(6) antibody was assessed in an L929 cell competition assay in which the antibody competes against the TNF receptor on L929 cells for binding to TNF in solution. In this assay the gL221/gH341(6) antibody was approximately 10% as active as murine CB0006.

#### 5     CB0010 (also known as hTNF1)

CB0010 is a monoclonal antibody which recognises an epitope of human TNF- $\alpha$ . The EU human framework was used for CDR-grafting of both the heavy and light variable domains. The amino acid sequences of the heavy and light variable domains of EU (EU), CB0010 (*h t n f 1*) and grafted versions of CB0010 (gEU, light; 2hEUg, heavy) are shown in Figure 1.

#### 10     Heavy Chain

In the CDR-grafted heavy chain mouse CDRs were used at positions 26-35 (CDR1), 50-65 (CDR2) and 95-102 (CDR3). Mouse residues were also used in the frameworks at positions 48, 67, 69, 71, 73, 76, 89, 91, 94 and 108. Comparison of the TNF1 mouse and EU human heavy chain residues reveals that these are identical at positions 23, 24, 29 and 78.

#### 20     Light Chain

In the CDR-grafted light chain mouse CDRs were used at positions 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3). In addition mouse residues were used in the frameworks at positions 3, 42, 48, 49, 83, 106 and 108. Comparison of the hTNF1 mouse and EU human light chain residues reveals that these are identical at positions 46, 58 and 71.

The grafted CB0010 heavy chain was co-expressed with the chimeric light chain and the binding ability of the product compared with that of the chimeric light chain/chimeric heavy chain product in a TNF binding assay. The grafted heavy chain product appeared to have binding ability for TNF slightly better than the fully chimeric product.

Similarly, a grafted heavy chain/grafted light chain product was co-expressed and compared with the fully chimeric product and found to have closely similar binding properties to the latter product. However when the grafted heavy chain/grafted light chain product was assayed in the L929 assay (see Example 4), it was found to have an activity only half that of the chimeric product. Thus further CDR-grafting experiments were carried out as described in Example 2.

#### 30     hTNF3

hTNF3 recognises an epitope on human TNF- $\alpha$ . The sequence of hTNF3 shows only 21 differences compared to CB0006 in the light and heavy chain variable regions, 10 in the light chain (2 in the CDRs at positions 50, 96 and 8 in the framework at 1, 19, 40, 45, 46, 76, 103 and 106) and 11 in the heavy chain (3 in the CDR regions at positions 52, 60 and 95 and 8 in the framework at 1, 10, 38, 40, 67, 73, 87 and 105). The light and heavy chain variable domain amino acid sequences of hTNF3 (Htnf3, light; hTNF3, heavy), CDR-grafted hTNF3 (gHTNF3, light; ghTNF3, heavy) and REI (REI, light) and KOL (KOL, heavy) are shown in Figure 4. The light and heavy chains of the CB0006 and hTNF3 chimeric antibodies can be exchanged without loss of activity in the direct binding assay. However CB0006 is an order of magnitude less able to compete with the TNF receptor on L929 cells for TNF- $\alpha$  compared to hTNF3. Based on the CB0006 CDR grafting data gL221 and gH341(+23, 24, 48, 49 71 and 73 as mouse) genes have been built for hTNF3 and tested and the resultant grafted antibody binds well to TNF- $\alpha$ , but competes very poorly in the L929 assay. The gL221 gene codes for the gHTNF3 and the gH341 etc. gene codes for the ghTNF3 variable domain sequences as shown in Figure 4. It is likely that in this case other framework residues may need to be changed to improve the competitive binding ability of this antibody.

#### 45     101.4

101.4 is a further murine MAb able to recognise human TNF- $\alpha$ . The heavy chain of this antibody shows good homology to KOL and so the CDR-grafting has been based on RE1 for the light chain and KOL for the heavy chain. The heavy and light variable domain amino acid sequences of 101.4 (101/4) and a CDR-grafted version of 101.4 (g1014) and the REI light chain (*1 r e i*) and KOL heavy chain (KOL) variable domains are given in Figure 2. Several grafted heavy chain genes have been constructed with conservative choices for the CDR's (gH341) and which have one or a small number of non-CDR residues at positions 73, 78 or 77-79 inclusive, as the mouse amino acids. These have been co-expressed with the chimeric light chain or the Kabat CDR-grafted light chain. In all cases binding to TNF equivalent to the chimeric antibody is seen and when co-expressed with cL the resultant antibodies are able to compete well in the L929 assay. However, with gL221 the resultant antibodies are at least an order of magnitude less able to compete for

TNF against the TNF receptor on L929 cells.

Mouse residues at other positions in the heavy chain, for example, at 23 and 24 together or at 76 have been demonstrated to provide no improvement to the competitive ability of the grafted antibody in the L929 assay.

## 5 Example 2

### Further CDR-Grafting of Murine anti-human TNF $\alpha$ Antibodies CB0010 and 101.4

10 Murine anti-human TNF $\alpha$  monoclonal antibodies CB0010 and 101.4 were further CDR-grafted substantially as described in WO91/09667.

#### CB0010

15 CB0010 is a monoclonal antibody which recognises an epitope on human TNF- $\alpha$ . The EU human framework was used for CDR-grafting of both the heavy and light variable domains.

The amino acid sequences of the heavy and light chain variable domains of the EU acceptor, CB0010 (*h t n f 1*) murine donor and CDR-grafted (gEU, light chain and 2hEUg, heavy chain) antibodies are given in Figure 1.

#### Heavy chain

20 In the CDR-grafted heavy chain (2hEUg), mouse CDRs were used at positions 31-35 (CDR1), 50-65 (CDR2) and 95-102 (CDR3).

25 Mouse residues were also used in the frameworks at positions 12, 27, 30, 38, 46, 48, 66, 67, 69, 71, 73, 76, 83, 89, 91, 94 and 108. Comparison of the CB0010 mouse and EU human heavy chain residues reveals that these are identical as positions 23, 24, 29 and 78.

#### Light chain

30 In the CDR-grafted light chain (gEU) mouse CDRs were used at positions 24-34 (CDR1), 50-65 (CDR2) and 89-97 (CDR3). In addition mouse residues were used in the frameworks at positions 3, 42, 48, 49, 106 and 108. The human consensus residue (valine) was used at position 83. Comparison of the CB0010 mouse and EU human light chain residues reveals that these are identical at positions 46, 58 and 71.

35 The grafted CB0010 heavy chain was co-expressed with the chimeric light chain and the binding ability of the product compared with that of the chimeric light chain/chimeric heavy chain product in a TNF binding assay. The grafted heavy chain product appeared to have binding ability for TNF slightly better than the fully chimeric product.

40 Similarly, a grafted heavy chain/granted light chain product was co-expressed and compared with the fully chimeric product and found to have closely similar binding properties to the latter product. The specific combination of grafted light chain (gEU) and grafted heavy chain (2hEUg), as shown in Figure 1, provides the antibody known as CDP571. The murine CB0010 (CB0010), chimeric CB0010 (chimeric CB0010) and the grafted heavy chain/granted light chain product (CDP571) were compared for binding to human TNF $\alpha$  in a standard assay. The results obtained are given in the table below in terms of the  $K_D$  (pM) measured for each antibody.

Antibody	$K_D$ (pM)
CB0010	80
Chimeric CB0010	81
CDP571	87

50 The fully grafted antibody product (CDP571) is currently in pre-clinical development for treatment of sepsis syndrome and acute transplant rejection.

#### 101.4

55 101.4 is a further murine MAb able to recognise human TNF- $\alpha$ . The heavy chain of this antibody shows good homology to KOL and so the CDR-grafting has been based on REI for the light chain and KOL for the heavy chain. An improved CDR-grafted product has been prepared. Variable domain amino acid sequences for REI (rei, light chain), KOL (KOL, heavy chain) murine 101.4 (101/4, heavy and light chain) and fully grafted antibody (g1014, heavy and light chain) are shown in Figure 2.

Heavy chain

In the CDR-grafted heavy chain (g1014) mouse CDRs were used at position 31-35 (CDR1), 50-65 (CDR2) and 95-102 (CDR3). Mouse residues were also used in the framework at positions 4, 11, 23, 24, 28, 73, 77, 78, 79, 91, 93, 94 and 108.

Light chain

In the CDR-grafted light chain (g1014) mouse CDRs were used at positions 24-34 (CDR1), 50-56 (CDR2) and 89-97 (CDR3). In addition mouse residues were used in the framework at positions 1, 3, 4, 39, 73, 105 and 107. The human consensus residue (valine) was used at position 104.

The fully grafted heavy and light chain (g1014) were co-expressed and their binding to TNF compared with murine and chimeric 101.4 and also the fully grafted (gEU/2hEUg, CDP571) CB0010 antibody. The fully grafted 101.4 antibody was found to having binding properties for human TNF $\alpha$  similar to the murine, chimeric, and grafted CB0010 antibodies.

Example 3In vitro comparison of Murine and CDR-grafted AntibodiesA. Affinity Measurements for Murine CB0010 and CDP571Materials and MethodsMaterials:

PBS/BSA: Dulbeccos PBS + 1% (w/v) bovine serum albumin.

TNF: 50nM rec. human TNF- $\alpha$  (Bissendorf Biochemicals), 0.85 $\mu$ g/ml in PBS/BSA.

Stock  $^{125}$ I-TNF: 5 $\mu$ Ci, 185kBq (Amersham International) dissolved in 500 $\mu$ l water and stored at -70°C.

Working Solution  $^{125}$ I-TNF: -62pM for titration curve and 124pM for Scatchard analysis, in PBS/BSA.

Antibodies: Purified murine CB0010 (mHTNF1) and CDP571 were quantified by A280nm ( $E_{1\text{mg/ml}}^{280\text{nm}}=1.4$ ), and diluted to a concentration of 1 $\mu$ g/ml for titration, or 200ng/ml for Scatchard analysis.

Immunobeads: Goat anti-murine IgG whole molecule-agarose or goat anti-human IgG whole molecule-agarose (Sigma) were used undiluted.

Method:

Antibody titration: mHTNF1 and CDP571 were titrated in doubling dilutions (100 $\mu$ l each) to give a total of 16 samples and  $^{125}$ I-TNF (100 $\mu$ l, 62pM) was added. The final top concentration of antibody was 500ng/ml and  $^{125}$ I-TNF was 31pM. Control tubes (8) contained  $^{125}$ I-TNF and PBS/BSA only. The samples were left to equilibrate overnight at room temperature, with shaking. After equilibration, 25 $\mu$ l goat anti-mouse-agarose was added to the mHTNF1 samples, and 50 $\mu$ l goat anti-human beads were added to the CDP571 samples except for the total  $^{125}$ I-TNF controls. Non-specific absorption of  $^{125}$ I-TNF to the agarose beads was corrected for by adding beads to 4 of the controls and comparing supernatant counts for these samples with those containing PBS/BSA instead of beads. After 1 hour equilibration at room temperature PBS/BSA (0.5ml) was added and the samples were centrifuged at 1500rpm for 10 mins at 20°C. The supernatant (0.5ml) was removed and radioactivity was counted in a gamma counter.

Confirmation that  $^{125}$ I-TNF behaved similarly to the unlabelled material in this assay was made by performing the antibody titration in the presence of mixtures of  $^{125}$ I-TNF and unlabelled TNF (at 25% and 75%  $^{125}$ I-TNF) at the same total concentration.

Scatchard analysis: For both antibodies, unlabelled TNF (100 $\mu$ l, 50nM) was titrated in duplicate, in 13 doubling dilutions. One sample containing PBS/BSA in place of TNF was included for each antibody.  $^{125}$ I-TNF (50 $\mu$ l, 124pM) was added to each sample. A constant amount of antibody, determined from the titration curve (50 $\mu$ l, 200ng/ml) was then added. This gave the following final concentrations: antibody, 50ng/ml; TNF, 25nM top concentration;  $^{125}$ I-TNF, 31pM. The samples were left to equilibrate overnight and then treated exactly as for the antibody titration samples.

## Calculations

### Titration Curves

5 Bound  $^{125}\text{I}$ -TNF cpm = NSB cpm - supernatant cpm

$$\frac{\text{Bound } ^{125}\text{I-TNF cpm}}{\text{Total } ^{125}\text{I-TNF}} = \text{B/T}$$

NSB = non-specific absorption blank, supernatant cpm

10 Total = total counts for  $^{125}\text{I}$ -TNF only B/T was plotted against antibody concentration and the appropriate antibody concentration for use in Scatchard analyses was chosen at B/T = 0.6

### Scatchard analysis

15 The mean of duplicate determination was used throughout

NSB = Total cpm - NSB supernatant cpm

Free cpm = sample cpm + NSB

Proportion of free TNF = Free/Total (F/T) =

$$\frac{\text{sample cpm} + \text{NSB cpm}}{\text{Total cpm}} = \text{B/F} = \frac{1 - \text{F/T}}{\text{F/T}}$$

B/F was plotted against Bound TNF to give a slope of  $-1/K_d$  from which  $K_d$  was calculated

## RESULTS

25 Dissociation constants for murine CB0010 and CDP571

Antibody	$K_d$ .M
Murine HTNF1	$1.3 \times 10^{-10}$
CDP571	$1.4 \times 10^{-10}$

30 B. Competition of murine CB0010 (MuhTNF1) and CDP571 (GrhTNF1) with HRP-conjugated in Murine CB0010 for binding to rhuTNF

### Method

A 96 well microtitre plate (Nunc, Maxisorb) was coated with 100 $\mu\text{l}$ /well TNF at 0.5 $\mu\text{g}/\text{ml}$ .

Serial dilutions of murine or grafted antibody were prepared using PBS/1% BSA diluent, from 200 $\mu\text{g}/\text{ml}$  to 0.01 $\mu\text{g}/\text{ml}$ . 40 50 $\mu\text{l}$  of antibody was added to each well followed by 50 $\mu\text{l}$  HRP-murine CB0010 at 3 concentrations (0.625, 0.315 and 0.16 $\mu\text{g}/\text{ml}$ ). Plates were incubated for 2 hours at room temperature with agitation, washed 4 times with PBS and 100 $\mu\text{l}$  of TMB substrate added.

Optical Density was measured and OD plotted against antibody concentration.

### Conclusions

45 Curves for both murine antibody (MuhTNF1) and grafted antibody (GrhTNF1) are superimposable, indicating both antibodies compete with similar affinity for binding to TNF (see Figure 5).

### Example 4

## Comparison of Murine CB0010 and CDR-grafted CDP571 Antibodies in Bioassay and Animal Model Experiments

### A. Neutralisation of TNF by CB0010 and CDP571 in the L929 Assay

55 The ability of the parent murine antibody CB0010 (hTNFI) and the CDR-grafted antibody CDP571 to neutralise recombinant human TNF was determined using the L929 bioassay. The assay uses the L929 mouse fibroblastoid cell

line which is killed by TNF. The assay is performed in the presence of 1 ug/ml actinomycin D which renders the cells more sensitive to TNF. Serial dilution of the two antibodies were mixed with a constant amount of recombinant human TNF (100 pg/ml) and added to a L929 monolayer in 96 well flat bottomed plates. After a 16 hour incubation the cells which had not been killed by TNF were revealed using the stain crystal violet. The apparent amount of TNF not neutralised (residual TNF) was determined by comparison with a recombinant TNF standard curve. Results from a representative experiment where residual TNF is plotted against antibody concentration are shown in Figure 6. It can be seen that CB0010 and CDP571 have similar neutralisation activities.

#### B. Effect of CDP571 in Baboon Sepsis Model

In this study the effect of the prior treatment with CDP571 on the physiological consequences of severe sepsis (including death) was assessed. Baboons were chosen as a relevant species to study since CDP571 is known to neutralise baboon TNF.

Male adult baboons, Papio ursinus, weighting 20-25 kg were anaesthetised with ketamine hydrochloride and sodium pentobarbitone and instrumented for the measurement of blood pressure, cardiac index (by thermodilution), ECG and right atrial filling pressures. An infusion of either saline only or antibody was then given for 120 min at a rate of 2.5 ml/kg/h following which they were given a further 120 min infusion of live E.coli at the same infusion rate. The bacterial strain used was Hinshaw's strain B7 ([086a:61], ATCC 33985) administered whilst in the log growth phase at a dose of  $2 \times 10^9$  CFU/kg giving a plasma concentration of  $2-2.5 \times 10^5$  CFU/ml at the end of the infusion. Following a further 120 min, animals were returned to their home cages, given free access to food and water and monitored for cardiovascular changes twice a day for 3 days. All animals were given constant fluid replacement infusion of 5 ml/kg/h which was adjusted, where necessary, to maintain adequate right heart filling pressures. Baboons that had died during treatment or that had survived the 72h experimental period, and then killed were post-mortemed. All major organs were assessed for gross macro-pathological damage according to semi-quantitative scale (+++ being the most severe).

Animals were randomly assigned to one of 4 treatment groups;

- saline only
- CDP571 0.1 mg/kg
- CDP571 1.0 mg/kg
- CB0010 0.1 mg/kg (parent murine antibody)

The survival and cumulative organ damage scores are shown in table 1. CDP571 at 1.0 mg/kg prevented death and significantly ( $P < 0.005$ ) reduced the incidence of organ damage in this model; furthermore, these effects were dose-related ( $P < 0.005$ ). In addition, the survival rate and organ damage score seen with CB0010 were similar to those seen with CDP571 at the same dose, indicating a maintained in vivo potency of CDP571 compared to its parent murine antibody.

Table 1

# BABOON SEPSIS STUDY

SURVIVAL FOLLOWING ADMINISTRATION OF 2X10<sup>9</sup> CFU E.Coli  
GIVEN IV 2H AFTER SALINE OR CDP 571

<u>TREATMENT</u>	<u>No</u>	<u>DEAD</u>	<u>SURVIVED</u>	<u>PERCENT SURVIVAL</u>	<u>ORGAN PATHOL.</u>
SALINE	8	7	1	13	+++
CDP571 0.1 mg/kg	6	2	4	67	++
CDP571 1.0 mg/kg	6	0	6	100	+/-
CB0010 0.1 mg/kg	4	1	3	75	++

References

1. Kohler & Milstein, Nature, 265, 295-497, 1975.
2. Chatenoud et al, (1986), J. Immunol. 137, 830-838.
3. Jeffers et al, (1986), Transplantation, 41, 572-578.
4. Begent et al, Br. J. Cancer 62: 487 (1990).
5. Verhoeyen et al, Science, 239, 1534-1536, 1988.
6. Riechmann et al, Nature, 332, 323-324, 1988.
7. Kabat, E.A., Wu, T.T., Reid-Miller, M., Perry, H.M., Gottesman, K.S., 1987, in Sequences of Proteins of Immunological Interest, US Department of Health and Human Services, NIH, USA.
8. Wu, T.T., and Kabat, E.A., 1970, J. Exp. Med. 132 211-250.
9. Queen et al, (1989), Proc. Natl. Acad. Sci. USA, 86, 10029-10033 and WO 90/07861
10. Tempest et al, (1991), Biotechnology, 9, 266-271
11. Beutler et al, (1985), Science, 234, 470-474
12. Maniatis et al, Molecular Cloning, Cold Spring Harbor, New York, 1989.
13. Primrose and Old, Principles of Gene Manipulation, Blackwell, Oxford, 1980.
14. Jones et al, (1986), Nature, 321, 522.

**Claims**

1. An antibody molecule which has specificity for human TNF $\alpha$  comprising a composite heavy chain and a complementary light chain, said composite heavy chain having a variable domain comprising predominantly human acceptor antibody heavy chain framework residues and donor antibody heavy chain antigen-binding residues, said donor antibody having specificity for said human TNF $\alpha$ , wherein, according to the Kabat numbering system, in said composite heavy chain, amino acid residues 23, 24, 31 to 35, 49 to 65 and 95 to 100 at least are donor residues.
2. The antibody molecule of claim 1, wherein amino acid residues 71, 73 and 78 in said composite heavy chain are additionally donor residues.
3. The antibody molecule of claim 1 or claim 2, wherein amino acid residues 6, 37, 48 and 94 are additionally donor residues.
4. The antibody molecule of any one of claims 1 to 3, wherein amino acid residues 2, 4, 25, 36, 39, 47, 93, 103, 104, 106 and 107 are additionally donor residues.
5. The antibody molecule of any one of claims 1 to 4, wherein amino acid residues 26 to 30 are additionally donor residues.
6. An antibody molecule which has specificity for human TNF $\alpha$  comprising a composite light chain and a complementary heavy chain, said composite light chain having a variable domain comprising predominantly human acceptor antibody light chain framework residues and donor antibody light chain antigen-binding residues, said donor antibody having specificity for said human TNF $\alpha$ , wherein, according to the Kabat numbering system, in said composite

light chain, amino acid residues 24 to 34, 46, 47, 50 to 56 and 89 to 97 at least are donor residues.

7. An antibody molecule which has specificity for human TNF $\alpha$  comprising a composite light chain and a complementary heavy chain, said composite light chain having a variable domain comprising predominantly human acceptor antibody light chain framework residues and donor antibody light chain antigen-binding residues, said donor antibody having specificity for said human TNF $\alpha$ , wherein, according to the Kabat numbering system, in said composite light chain, amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97 at least are donor residues.
8. The antibody molecule of any one of claims 1 to 5, wherein the complementary light chain is a composite light chain, said composite light chain having a variable domain comprising predominantly human acceptor antibody light chain framework residues and donor antibody light chain antigen-binding residues, wherein, according to the Kabat numbering system, in said composite light chain, amino acid residues 24 to 34, 46, 47, 50 to 56 and 89 to 97 at least are donor residues.
9. The antibody molecule of any one of claims 1 to 5, wherein the complementary light chain is a composite light chain, said composite light chain having a variable domain comprising predominantly human acceptor antibody light chain framework residues and donor antibody light chain antigen-binding residues wherein, according to the Kabat numbering system, in said composite light chain, amino acid residues 24 to 34, 46, 48, 50 to 56, 58, 71 and 89 to 97 at least are donor residues.
10. The antibody molecule of any one of claims 6 to 9, wherein amino acid residues 2, 4, 6, 35, 36, 38, 44, 47, 49, 62, 64 to 69, 85, 87, 98, 99, 101 and 102 in said light chain are additionally donor residues.
11. The antibody molecule of claim 10, wherein amino acid residues 1, 3, 10 to 12, 21, 37, 40, 45, 60, 63, 70, 73, 83, 103 and 105 in said light chain are additionally donor residues.
12. A therapeutic composition comprising the antibody molecule of any one of claims 1 to 11 in combination with a pharmaceutically acceptable carrier.
13. The antibody molecule of any one of claims 1 to 12 or the therapeutic composition of claim 12 for use in a therapeutic method comprising administering the antibody to a human subject in an amount effective to neutralize TNF $\alpha$ .
14. The antibody molecule of any one of claims 1 to 12 or the therapeutic composition of claim 12 for use in treating rheumatoid arthritis.

#### Patentansprüche

1. Ein Antikörpermolekül mit Spezifität für humanen TNF $\alpha$ , welches eine zusammengesetzte schwere Kette und eine komplementäre leichte Kette umfaßt, wobei die zusammengesetzte schwere Kette einen variablen Bereich hat, der hauptsächlich Rahmenreste einer schweren Kette eines humanen Akzeptor-Antikörpers und Antigenbindungsreste einer schweren Kette eines Donor-Antikörpers umfaßt und der Donor-Antikörper Spezifität für den humanen TNF $\alpha$  hat, in welchem Antikörpermolekül gemäß dem Kabat-Numerierungssystem in der genannten zusammengesetzten schweren Kette zumindest die Aminosäurereste 23, 24, 31 bis 35, 49 bis 65 und 95 bis 100 Donor-Reste sind.
2. Das Antikörpermolekül nach Anspruch 1, in welchem die Aminosäurereste 71, 73 und 78 in der genannten zusammengesetzten schweren Kette zusätzlich Donor-Reste sind.
3. Das Antikörpermolekül nach Anspruch 1 oder Anspruch 2, in welchem die Aminosäurereste 6, 37, 48 und 94 zusätzlich Donor-Reste sind.
4. Das Antikörpermolekül nach einem der Ansprüche 1 bis 3, in welchem die Aminosäurereste 2, 4, 25, 36, 39, 47, 93, 103, 104, 106 und 107 zusätzlich Donor-Reste sind.
5. Das Antikörpermolekül nach einem der Ansprüche 1 bis 4, in welchem die Aminosäurereste 26 bis 30 zusätzlich Donor-Reste sind.
6. Ein Antikörpermolekül mit Spezifität für humanen TNF $\alpha$ , das eine zusammengesetzte leichte Kette und eine kom-



plementaire schwere Kette umfaßt, wobei die zusammengesetzte leichte Kette einen variablen Bereich hat, der hauptsächlich Rahmenreste einer leichten Kette eines humanen Akzeptor-Antikörpers und Antigenbindungsreste einer leichten Kette eines Donor-Antikörpers umfaßt und der Donor-Antikörper Spezifität für den humanen TNF $\alpha$  hat, in welchem Antikörpermolekül gemäß dem Kabat-Numerierungssystem in der genannten zusammengesetzten leichten Kette zumindest die Aminosäurereste 24 bis 34, 46, 47, 50 bis 56 und 89 bis 97 Donor-Reste sind.

7. Ein Antikörpermolekül mit Spezifität für humanen TNF $\alpha$ , das eine zusammengesetzte leichte Kette und eine komplementäre schwere Kette umfaßt, wobei die zusammengesetzte leichte Kette einen variablen Bereich hat, der hauptsächlich Rahmenreste einer leichten Kette eines humanen Akzeptor-Antikörpers und Antigenbindungsreste einer leichten Kette eines Donor-Antikörpers umfaßt und der Donor-Antikörper Spezifität für den humanen TNF $\alpha$  hat, in welchem Antikörpermolekül gemäß dem Kabat-Numerierungssystem in der genannten zusammengesetzten leichten Kette zumindest die Aminosäurereste 24 bis 34, 46, 48, 50 bis 56, 58, 71 und 89 bis 97 Donor-Reste sind.
8. Das Antikörpermolekül nach einem der Ansprüche 1 bis 5, in welchem die komplementäre leichte Kette eine zusammengesetzte leichte Kette ist, die einen variablen Bereich hat, der hauptsächlich Rahmenreste einer leichten Kette eines humanen Akzeptor-Antikörpers und Antigenbindungsreste einer leichten Kette eines Donor-Antikörpers umfaßt, in welchem Antikörpermolekül gemäß dem Kabat-Numerierungssystem in der genannten zusammengesetzten leichten Kette zumindest die Aminosäurereste 24 bis 34, 46, 47, 50 bis 56 und 89 bis 97 Donor-Reste sind.
9. Das Antikörpermolekül nach einem der Ansprüche 1 bis 5, in welchem die komplementäre leichte Kette eine zusammengesetzte leichte Kette ist, die einen variablen Bereich hat, der hauptsächlich Rahmenreste einer leichten Kette eines humanen Akzeptor-Antikörpers und Antigenbindungsreste einer leichten Kette eines Donor-Antikörpers umfaßt, in welchem Antikörpermolekül gemäß dem Kabat-Numerierungssystem in der genannten zusammengesetzten leichten Kette zumindest die Aminosäurereste 24 bis 34, 46, 48, 50 bis 56, 58, 71 und 89 bis 97 Donor-Reste sind.
10. Das Antikörpermolekül nach einem der Ansprüche 6 bis 9, in welchem die Aminosäurereste 2, 4, 6, 35, 36, 38, 44, 47, 49, 62, 64 bis 69, 85, 87, 98, 99, 101 und 102 in der genannten leichten Kette zusätzlich Donor-Reste sind.
11. Das Antikörpermolekül nach Anspruch 10, in welchem die Aminosäurereste 1, 3, 10 bis 12, 21, 37, 40, 45, 60, 63, 70, 73, 83, 103 und 105 in der genannten leichten Kette zusätzlich Donor-Reste sind.
12. Eine therapeutische Zusammensetzung, die das Antikörpermolekül nach einem der Ansprüche 1 bis 11 in Kombination mit einem pharmazeutisch verwendbaren Träger umfaßt.
13. Das Antikörpermolekül nach einem der Ansprüche 1 bis 11 oder die therapeutische Zusammensetzung nach Anspruch 12 zur Verwendung in einem therapeutischen Verfahren, bei welchem der Antikörper in einer zur Neutralisation von TNF $\alpha$  wirksamen Menge an einen Menschen verabreicht wird.
14. Das Antikörpermolekül nach einem der Ansprüche 1 bis 11 oder die therapeutische Zusammensetzung nach Anspruch 12 zur Verwendung bei der Behandlung von rheumatoider Arthritis.

## Revendications

1. Molécule d'anticorps qui présente une spécificité vis à vis du FNT- $\alpha$  humain, comprenant une chaîne lourde composite et une chaîne légère complémentaire, ladite chaîne lourde composite ayant un domaine variable comprenant d'une manière prédominante des résidus de la région charpente d'une chaîne lourde d'anticorps accepteur humain et des résidus se liant à l'antigène d'une chaîne lourde d'anticorps donneur, ledit anticorps donneur présentant une spécificité vis à vis dudit FNT- $\alpha$  humain, où, conformément au système de numérotation de Kabat, dans ladite chaîne lourde composite, les résidus d'acides aminés 23, 24, 31 à 35, 49 à 65 et 95 à 100, au moins, sont des résidus du donneur.
2. Molécule d'anticorps selon la revendication 1, où les résidus d'acides aminés 71, 73 et 78 dans ladite chaîne lourde composite sont en outre des résidus du donneur.
3. Molécule d'anticorps selon la revendication 1 ou la revendication 2, où les résidus d'acides aminés 6, 37, 48 et 94 sont en outre des résidus du donneur.

4. Molécule d'anticorps selon l'une quelconque des revendications 1 à 3, où les résidus d'acides aminés 2, 4, 25, 36, 39, 47, 93, 103, 104, 106 et 107 sont en outre des résidus du donneur.
- 5 5. Molécule d'anticorps selon l'une quelconque des revendications 1 à 4, où les résidus d'acides aminés 26 à 30 sont en outre des résidus du donneur.
- 10 6. Molécule d'anticorps qui présente une spécificité vis à vis du FNT- $\alpha$  humain, comprenant une chaîne légère composite et une chaîne lourde complémentaire, ladite chaîne légère composite ayant un domaine variable comprenant de manière prédominante des résidus de la région charpente d'une chaîne légère d'anticorps accepteur humain et des résidus se liant à l'antigène d'une chaîne légère d'anticorps donneur, ledit anticorps donneur présentant une spécificité vis à vis dudit FNT- $\alpha$  humain, où, conformément au système de numérotation de Kabat, dans ladite chaîne légère composite, les résidus d'acides aminés 24 à 34, 46, 47, 50 à 56 et 89 à 97, au moins, sont des résidus du donneur.
- 15 7. Molécule d'anticorps qui présente une spécificité vis à vis du FNT- $\alpha$  humain, comprenant une chaîne légère composite et une chaîne lourde complémentaire, ladite chaîne légère composite ayant un domaine variable comprenant de manière prédominante des résidus de la région charpente d'une chaîne légère d'anticorps accepteur humain et des résidus se liant à l'antigène d'une chaîne légère d'anticorps donneur, ledit anticorps donneur présentant une spécificité vis à vis dudit FNT- $\alpha$  humain, où, conformément au système de numérotation de Kabat, dans ladite chaîne légère composite, les résidus d'acides aminés 24 à 34, 46, 48, 50 à 56, 58, 71 et 89 à 97, au moins, sont des résidus du donneur.
- 20 8. Molécule d'anticorps selon l'une quelconque des revendications 1 à 5, où la chaîne légère complémentaire est une chaîne légère composite, ladite chaîne légère composite ayant un domaine variable comprenant de manière prédominante des résidus de la région charpente d'une chaîne légère d'anticorps accepteur humain et des résidus se liant à l'antigène d'une chaîne légère d'anticorps donneur, où, conformément au système de numérotation de Kabat, dans ladite chaîne légère composite, les résidus d'acides aminés 24 à 34, 46, 47, 50 à 56 et 89 à 97, au moins, sont des résidus du donneur.
- 25 9. Molécule d'anticorps selon l'une quelconque des revendications 1 à 5, où la chaîne légère complémentaire est une chaîne légère composite, ladite chaîne légère composite ayant un domaine variable comprenant de manière prédominante des résidus de la région charpente d'une chaîne légère d'anticorps accepteur humain et des résidus se liant à l'antigène d'une chaîne légère d'anticorps donneur, où, conformément au système de numérotation de Kabat, dans ladite chaîne légère composite, les résidus d'acides aminés 24 à 34, 46, 48, 50 à 56, 58, 71 et 89 à 97, au moins, sont des résidus du donneur.
- 30 10. Molécule d'anticorps selon l'une quelconque des revendications 6 à 9, où les résidus d'acides aminés 2, 4, 6, 35, 36, 38, 44, 47, 49, 62, 64 à 69, 85, 87, 98, 99, 101 et 102 dans ladite chaîne légère sont en outre des résidus du donneur.
- 35 11. Molécule d'anticorps selon la revendication 10, où les résidus d'acides aminés 1, 3, 10 à 12, 21, 37, 40, 45, 60, 63, 70, 73, 83, 103 et 105 dans ladite chaîne légère sont en outre des résidus du donneur.
- 40 12. Composition thérapeutique comprenant la molécule d'anticorps selon l'une quelconque des revendications 1 à 11 en association avec un support acceptable d'un point de vue pharmaceutique.
- 45 13. Molécule d'anticorps selon l'une quelconque des revendications 1 à 11 ou composition thérapeutique selon la revendication 12, pour une utilisation dans une méthode thérapeutique comprenant l'administration à un être humain d'une quantité efficace d'anticorps pour neutraliser le FNT- $\alpha$ .
- 50 14. Molécule d'anticorps selon l'une quelconque des revendications 1 à 11 ou composition thérapeutique selon la revendication 12, pour une utilisation dans le traitement de la polyarthrite chronique évolutive.
- 55

CDR GRAFTING OF hTNF-1Light Chain Data

1 Eu DIQMTQSPST LSASVGDRVIT ITCRASQSI. ....NTWLA WYQOKPGKAPK  
 htnf1 DIMMSQSPSS LAVSVGEKVTMS CKSSQSLLYSNNQKNYLA WYQOKPGQSPK  
 g Eu DIMMTQSPST LSASVGDRVITIT CKSSQSLLYSNNQKNYLA WYQOKPGQAPK

Eu LLMYKASSLE SGVPSRFIGS GSGTEFTLTI SSLQPDDFAT YYCQQYNSDS  
 htnf1 LLISWASTRES GVPDRFTGS GSGTDFTLTI SSVKAEDLAV YYCQQYYDYP  
 gEu LLISWASTRES GVPDRFIGS GSGTEFTLTI SSLQPDDVAT YYCQQYYDYP

3 Eu KMFGQG TKVEVKG..(KAPPA)  
 htnf1 WTFGGG SKLEIK.....anti human TNF seq from  
 g Eu WTFGGG TKVEIKR..(KAPPA)

framework residues changed (# = Kabat)

chgs 3/42/48/49/83/106/108

Heavy Chain Data

Eu QVQLVQSGAE VKKPGSSVKV SCKASGGTFSRSALII WVRQA PGQGLEWMGG  
 htnf1 EVLLQQSGPE LVKPGASVKI PCKASGYTFTDYNVD WVKQS HGKSLQWIGN  
 2hEug QVQLVQSGAE VVKPGSSVKV SCKASGYTFTDYNVD WVKQA PGQGLQWIGN

Eu IVPFMFGPPNYAQKFQG RVTITADESTNTAYMELSSLRSED TAFYFCAGGY  
 htnf1 INPNNGGTIYNQKFKG KGTLTVDKSSSTAYMELRSLTSED TAVYYCARSA  
 2hEug INPNNGGTIYNQKFKG KGTLTVDKSTSTAYMELSSLTSED TAVYYCARSA

Eu GIYSPE WGQGLTVTVSS.grp 1kabat cdr chg frwk4  
 htnf1 FYNNEYFDV WGAGTTVTVSS  
 2hEug FYNNEYFDV WGQGLTVTVSS

framework residues changed (# = kabat)

chgs 12/27/30/38/46/48/66/67/69/71/73/76/83/89/91/94/108

Fig. 1

CDR GRAFTING OF 101-4LIGHT CHAIN SUMMARY

1 rei DIQMTQSPSS LSASVGDRVT ITCQASQDI. ....IKYLNW YQQTGKAPK  
 101/4 QIVLTQSPPI MSASPGEKVT MTCASASSVSFMY W YQQKPGSSPR  
 g1014 QIVLTQSPSS LSASVGDRVT ITCASASSVSFMY W YQQKPGKAPK

2 rei LLIYEASNLQA GVPSRFSGS GSGTDYTFTI SSLQPEDIAT YYCQQYQSLP  
 101/4 LLIYDASILAS GVPVRFSGS GSGTSYSLTI SRMEAEDVAT YYCQQWSDYS  
 g1014 LLIYDASILAS GVPSRFSGS GSGTDYTLTI SSLQPEDIAT YYCQQWSDYS

3 rei YTFGQGTKLQ ITR..celltech rei  
 101/4 PRTFGGGTKLE IKR.....THIS IS MOUSE(INSERTION IN CDR3)JSE  
 g1014 PRTFGQGTKVE IKR..celltech rei

framework residues changed (# = Kabat)

1/3/4/39/73/104/105/107

HEAVY CHAIN SUMMARY

23 48  
 KOL QVQLVESGGG VVQPGRSRLR SCSSSGFIFSSYAMY WVRQA PGKGLEWVAI  
 101/4 EVKIEESGGG WVQPGGSMKL SCIASGFTFSNYWMN WVRQS PEKGLEWVAE  
 g1014 QVQIVESGGG WVQPGRSRLR SCIASGFTFSNYWMN WVRQA PGKGLEWVAE

71 88  
 KOL IWDDGSDQHYADSVKG RFTISRDN SKNTLFLQMDSLRPED TGVYFCARDG  
 101/4 VRLQSDNFTTHYAESVKGRFTISRDDSKSGVYLQMNNLGAED TGIYYCTPFA  
 g1014 VRLQSDNFTTHYAESVKGRFTISRDDSKNGVYLQMDSLRPED TGVYYCTPFA

KOL GHGFCSSASCFGPDY WGQGTPVTVSS....HUMAN(kabat CDR defn)  
 101/4 Y WGQGTLVTVSP...MOUSE seq  
 g1014 Y WGQGTLVTVSS

framework residues changed (# = Kabat)

4/11/23/24/28/73/77/78/79/91/93/94/108

Fig. 2

61E71

LIGHT CHAIN SUMMARY

REI DIQMTQSPSS LSASVGDRVT ITCQASQDI. ....IKYLN WYQOTPGKAPK  
 CB6 SIVMTQTPKF LLVSAGDRVT ITCKASQSVS NDVA WYQQKSGQSPK  
 gCB6 DIQMTQSPSS LSASVGDRVT ITCKASQSVS NDVA WYQOTPGKAPK

REI LLIYEASNLQA GVPSRFSGS GSGTDYFTTI SSLQPEDIAT YYCQQYQSLP  
 CB6 VLIYHVSNNRYT GVPDRFTGS GYGTDFTTI TTVQAEDLAV YFCQQDYSSP  
 gCB6 LLIYHVSNNRYT GVPSRFSGS GSGTDYFTTI SSLQPEDIAT YYCQQDYSSP

REI YTFGQGTLQ ITR... celltech rei(KAPPA)  
 CB6 WTFGGGTLKLE IK.... MOUSE AB... REI (NO FWRKS)  
 gCB6 WTFGQGTLQ ITR... grafted AB sequence

HEAVY CHAIN SUMMARY

KOL QVQLVESGGG VVQPGRLRL SCSSSGFIFSSYAMY WVRQA PGKGLEWVAI  
 CB6 QIQLVQSGPD LKKPGETVKI SCKASGYTFTNYGMN WVKQT PGKGLKWMGW  
 gCB6 QVQLVESGGG VVQPGRLRL SCKASGYTFTNYGMN WVRQA PGKGLEWWMGW

KOL IWDDGSDQHYADSVKG RFTISRDNKNTLFLQMDSLRLPED TGVYFCARDG  
 CB6 INTYTGEPTYDDDFKG RPAPFSLASASTAYLQINNLIKED MATFFCARQE  
 gCB6 INTYTGEPTYDDDFKG RFTISLDASKNTLFLQMDSLRLPED TGVYFCARQE

KOL GHGFCSSASCFGPDY WGQGTPVTVS.HUMAN grp3(kabat CDR defn)  
 CB6 GFYAMDY WGQGTSLTVSS..MOUSE ANTI-TNF sequence  
 gCB6 GFYAMDY WGQGTPVTVS.grafted AB sequence

Fig. 3

## hTNF3

LIGHT CHAIN SUMMARY

REI DIQMTQSPSS LSASVGDRVT ITCQASQDI. ....IKYLN WYQQTPGKAPK  
 hTNF3 NIVMTQTPKF LLVSAGDRIT ITCKASQSVS NDVA WYQQKPGQSPR  
 ghTNF3 DIQMTQSPSS LSASVGDRVT ITCKASQSVS NDVA WYQQTPGKAPK  
  
 REI LLIYEASNLA GVPSTRFSGS GSGTDYFTI SSLQPEDIAI YYCQQYQSLP  
 hTNF3 LLIYYVSNRYT GVPDRFTGS GYGTDFFTI NTVQAEDLAY YFCQQDYSSP  
 ghTNF3 LLIYYVSNRYT GVPSTRFSGS GSGTDYFTI SSLQPEDIAI YYCQQDYSSP  
  
 REI YTFGQGTLQ ITR.. (KAPPA)  
 hTNF3 YTFGGGTRLE VK.... MOUSE AB sequence  
 ghTNF3 YTFGQGTLQ ITR..grafted sequence

HEAVY CHAIN SUMMARY

KOL QVQLVESGGG VVQPGSLRL SCSSSGFIFSSYAMY WVRQA PGKGLEWVAI  
 hTNF3 RIQLVQSGPE LKKPGETVKI SCKASGYFTNYGMN WVTQA PGKGLKWMGW  
 ghTNF3 QVQLVESGGG VVQPGSLRL SCKASGYFTNYGMN WVRQA PGKGLEWMGW  
  
 KOL IWDDGSDQHYADSVKG RFTISRDNSTLFLQMDSLRPED TGVYFCARDG  
 hTNF3 INTYTGEPTYADDFKG RFAFSLETSASTAYLQINNLTNED TATYFCARKE  
 ghTNF3 INTYTGEPTYADDFKG RFTISLDTSKNTLFLQMDSLRPED TGVYFCARKE  
  
 KOL GHGFCSSASCFGPDY WGQGTPVTVS.HUMAN grp3  
 hTNF3 GFYAMDY WGQGTSTVTVSS...MOUSE.ANTI-TNF sequence  
 ghTNF3 GFYAMDY WGQGTPVTVS.grafted AB sequence

Fig. 4

## hTNF1 COMP ASSAY

MuhTNF1 / GrhTNF1

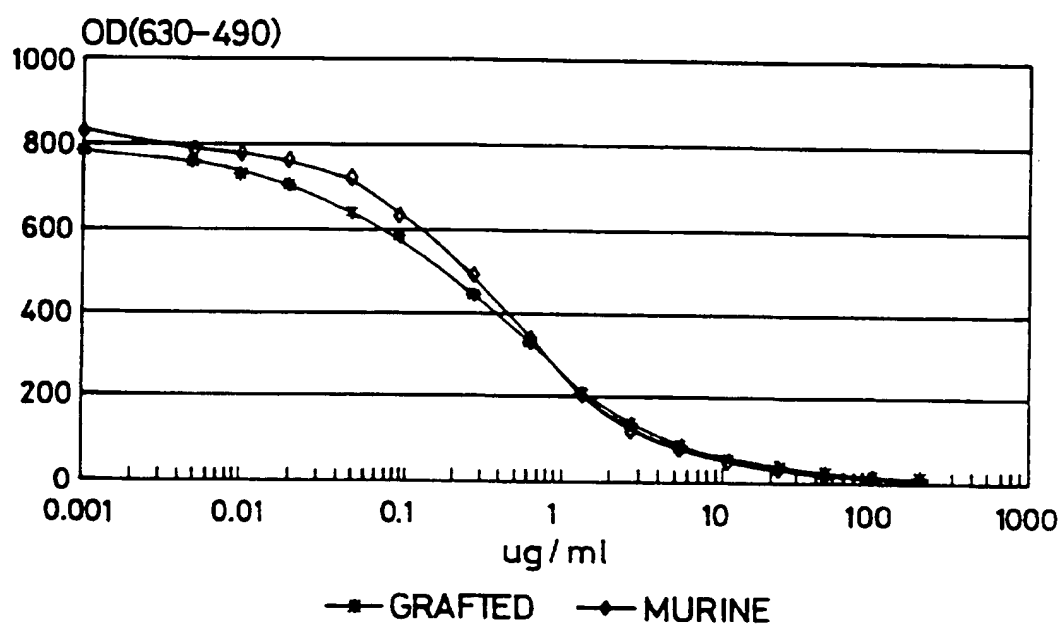


Fig. 5

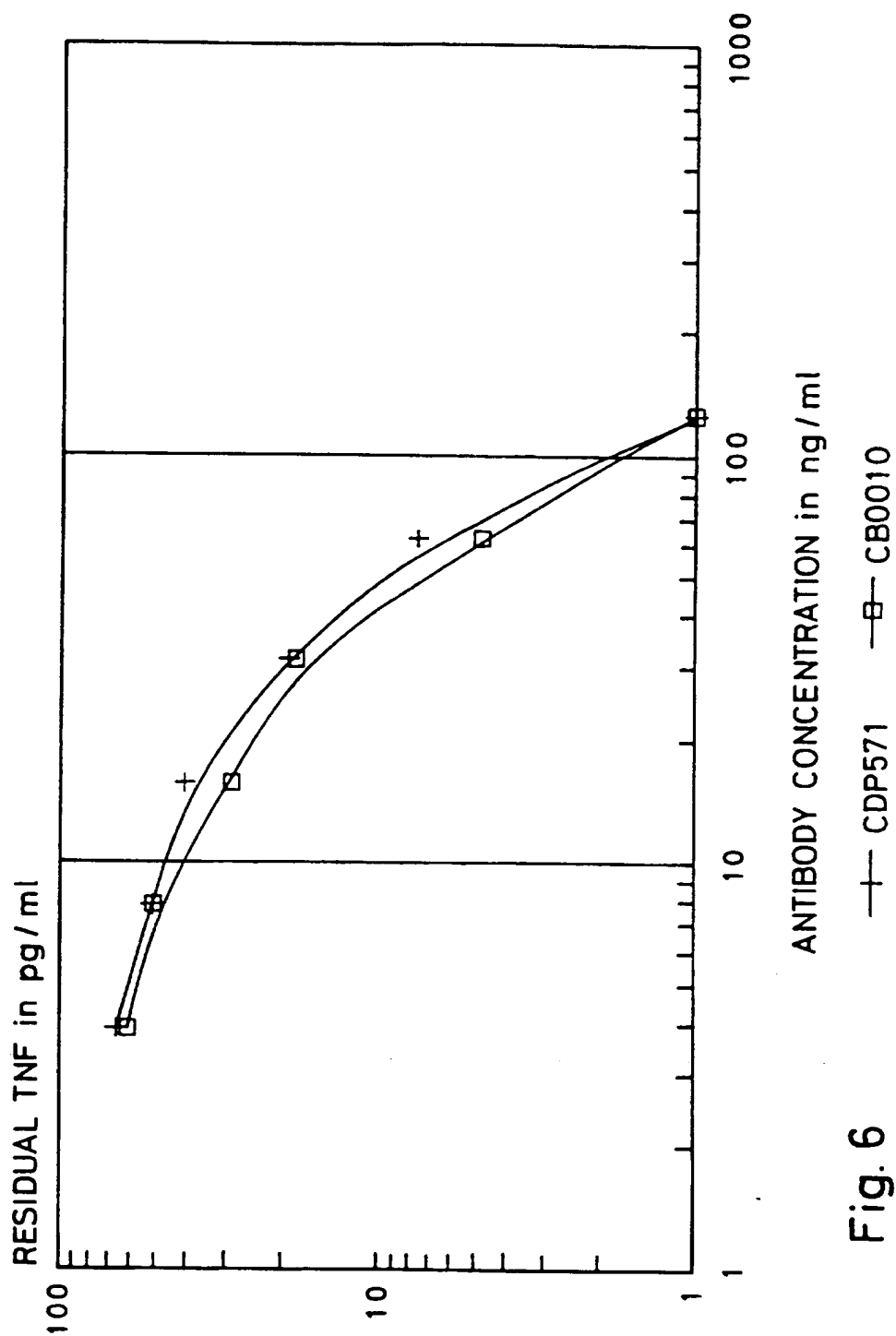
NEUTRALISATION OF 100 pg/ml RECOMBINANT  
TNF BY CB0010 AND CDP571

Fig. 6